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Phagotrophic ciliates and flagellates in an oligotrophic, deep, alpine lake: contrasting variability with seasons and depths

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ABSTRACT: We followed the changes in the protist assemblage over an annual cycle at 3 sites and different depths of Traunsee in the Austrian Alps and quantified the variability of the ciliate assemblage along successive depth and time intervals, respectively. More than 60 ciliate species were identified alive and after quantitative protargol staining (QPS). The ciliate diversity was high, and is described in detail for 50 taxa in time–depth intervals. *Rimostrombidium brachykinetum*/*Rimostrombidium hyalinum* and *Balanion planctonicum* were the most frequent species, accounting for 44% of the annual mean abundance and 15% of the annual mean biovolume, respectively. Our results suggested a stronger variability in the ciliate assemblage structure within seasons than along the depth gradient. Gradual changes in the assemblage structure with depths: (1) were accompanied by a decrease of algivorous and mixotrophic and an increase of bacterivorous ciliates from surface to deeper layers; (2) were highly significantly altered with steep depth gradients of their potential food resources, i.e. the biomass of heterotrophic flagellates, bacteria and algae; and (3) were related with lower significance to environmental parameters. High similarity (>80%) between successive months was reached only when net changes in the total ciliate abundance were negligible, while a strong increase or decrease in the ciliate abundance was associated with pronounced changes in the species composition. These seasonal changes in the ciliate assemblage structure (4) were linked to shifts of algivorous and mixotrophic, but not of bacterivorous ciliates and (5) were less predictable with food resources compared to the depth gradient. The phagotrophic flagellates generally followed the seasonal and vertical patterns described for ciliates, and were shown to be important members of the planktonic food web in a cold, deep, oligotrophic lake.

KEY WORDS: Microbial assemblage structure · Functional analysis · Microbial food web · Aquatic protists · Seasonal succession · Ciliate species list

INTRODUCTION

It is well known that protists (unicellular eukaryotes) play a pivotal role in the food web of oceans and lakes (e.g. Azam et al. 1983, Weisse & Müller 1998). In recent years, knowledge about single ciliate and flagellate species has accumulated, a prerequisite to understanding their contribution and importance to the cycling of carbon and nutrients (see Foissner et al. 1999 and references therein). Nonetheless, reliable data sets on planktonic protistan species are still rare, especially from oligotrophic lakes (Laybourn-Parry 1994, Pfister et al. 2002, Sonntag et al. 2002). In initial studies on planktonic protists, this lack was related to methodological difficulties in the determination of species present at low numbers, i.e. <1 ml^{-1}. This problem, however, has partly been solved by introduction of the Quantitative Protargol Stain (QPS;

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Montagnes & Lynn 1987, Skibbe 1994), which has been tested for its reliability (Pfister et al. 1999). The microbial community structure in aquatic ecosystems is variable both in time and space; however, few ecological studies have been explicitly devoted to analysis of the magnitude of both kinds of variability operating simultaneously and their underlying causes. In the present study, we focused on 2 major questions. (1) What factors — biotic and/or abiotic — govern the distribution of phagotrophic ciliates and flagellates in the water column of a deep, oligotrophic lake? (2) Does the ciliate assemblage structure correspond to the temporal and spatial distributions of its potential food resources? We interpreted changes in the protist assemblage from high taxonomic and spatial resolution data. According to literature (see below), single ciliate species were categorised according to their prevailing mode of nutrition, i.e. algivorous, mixotrophic, bacterivorous and omnivorous, and were analysed along a time and depth gradient. As potential food sources for the ciliates, we considered chlorophyll *a* (chl *a*), in different size fractions (see below), to be equivalent to autotrophic prey organisms, heterotrophic bacteria and heterotrophic flagellates. Bacteria were considered the main food for phagotrophic flagellates.

MATERIALS AND METHODS

As the study site (Table 1) and sampling design have already been described in detail (Klammer et al. 2002, Sonntag et al. 2002, Teubner & Dokulil 2002), we give only brief descriptions.

Investigation site. Traunsee is the second largest and deepest lake situated in the north of the Austrian limestone Alps. The lake has been influenced by the discharge from salt and soda industries for almost 100 yr. A study was carried out to investigate the impact of these wastes on organisms (Schmidt et al. 2002 and references therein). Results have shown that Traunsee receives 135000 tons of alkaline and saline industrial waste per year (Jagsch et al. 2002, Müller et al. 2002). The dissolved salts are mixed with lake water, with maximum concentrations in the hypolimnion, whereas solids are deposited in the southern part of the lake and have, in the meantime, covered ~20% of the lake bottom via turbidity currents (Müller et al. 2002). Benthic microorganisms in this southern part of Traunsee are heavily affected by their alkaline environment (pH > 9 in the pore water), e.g. bacterial abundance and biomass have been reduced by a factor of 10 compared to undisturbed sediment areas (Griebler et al. 2002). Several publications on the lake have focused on the effect of pollution from salt and soda industries and described, for example, microbial activities such as primary and bacterial production (Griebler et al. 2002, Klammer et al. 2002, Schmidt et al. 2002, Sonntag et al. 2002, Teubner & Dokulil 2002, Teubner 2003).

Monthly samples taken from November 1997 to October 1998 were analysed at 3 sites selected for a reference study on the microbial community with respect to the disposal of alkaline wastes (sampling map in Sonntag et al. 2002): (1) Viechtau (VI; the reference site at a maximum depth of 191 m), (2) Ebensee Bay (EB; the site of industrial waste release; depth 50 m), and (3) Rindbach Bay (RB; unpolluted area, influenced by allochthonous material from the main tributary, the Traun River; depth 50 m). Sampling depths were 0, 10, 20, 40 m at the 3 sites, and, additionally, 60, 80, 120 and 160 m at VI. Throughout the investigation period, Traunsee had a fully oxygenated water column down to the maximum depth (191 m) and increased chloride accumulation with depth. The lake is holomictic, and mixis occurred in February/March 1998.

Sample treatments and statistics. Oxygen, pH, conductivity, temperature and underwater light were measured immediately after sampling by sensors (WTW) and *in situ* by a profiler (multi-parameter profiler YSI 6920, 4π quantum sensor, LI-COR). Protocols

Table 1. Hydrological, morphometric and physico-chemical parameters of Traunsee. Temperature (T), pH, O_2, conductivity, chloride concentrations, total phosphorus (TP), chlorophyll *a* (chl *a*), mixing depth (z_{mix}) and euphotic zone (z_{eu}) are averages for the period November 1997 to October 1998, if not otherwise stated (modified from Pechlaner & Sossau 1982, Sonntag et al. 2002, Teubner & Dokulil 2002, Teubner 2003). Annual means are given with minimum and maximum values in brackets

Latitude	47°48′ to 47°55′N
Longitude	13°46′ to 13°49′E
Altitude (m a.s.l.)	422
Volume (10^9 m^3)	2.3
Lake surface area (km^2)	24.4
Catchment area (km^2)	1417
Maximum length (km)	12.2
Maximum width (km)	2.9
Maximum depth (m)	191
Mean depth (m)	95
z_{mix} (Jul–Aug) (m)	6.8
z_{eu} (m)	11.3 (7.6–17.6)
Water renewal time (yr)	1.04
Average discharge (m^3 s^{-1})	70
T (°C)	7.8 (4.2–20.2)
pH	8.4 (6.9–9.8)
O_2 (mg l^{-1})	10.6 (7.3–15.0)
Conductivity (µS cm^{-1})	536 (272–840)
Chloride (mg l^{-1})	95 (46–172)
TP (µg l^{-1})	7.7 (4–13.2)
Chl *a* (0–40 m) (µg l^{-1})	1.04 (0.14–3.3)

for the measurements of concentrations of chloride, total phosphorus, chlorophyll, phytoplankton composition and bacterial parameters are described in Griebler et al. (2002), Klammer et al. (2002), Teubner & Dokulil (2002) and Teubner (2003). Ciliates and flagellates were fixed immediately after sampling with either 5% Bouin's solution (final concentration) or 2% formaldehyde (final concentration), respectively. Living ciliates and flagellates were gathered for detailed taxonomic information with a 10 µm plankton net and observed within 24 h after sampling. Subsequently, ciliate samples were stained (with QPS in the modification of Pfister et al. 1999), and flagellates were stained with DAPI (4′,6-diamidino-2-phenylindole, Sigma; further details described in Porter & Feig [1980], Sherr & Sherr [1993] and Sonntag et al. [2000]). The protists were investigated with a Zeiss Axiophot II microscope (DIC, brightfield, phase contrast, epifluorescence) and identified morphologically after Foissner et al. (1991, 1992, 1994, 1995, 1999) and Patterson & Larsen (1991 and references therein). The microscopic permanent slides with the QPS-stained ciliates from this investigation were deposited and are available from the Oberösterreichische Landesmuseum in Linz (LI), Austria (www.biologiezentrum.at).

Fixed phagotrophic flagellates were measured by the use of a semi-automatic image analysis system (Lucia D V3.52 laboratory imaging software; for details see Sonntag et al. 2000). Ciliate dimensions were either measured on living specimens, if available, or on QPS-stained individuals with an eyepiece micrometer. Cell volumes of all protists were calculated from appropriate geometric shapes. Neither correction factors for biovolumes, nor conversion factors for biomasses were applied, as shrinkage/enlargement of ciliate cells is species specific and errors might increase (for details see Pfister et al. 1999). However, for the more-or-less uniform phagotrophic flagellates (see below), we applied the carbon conversion factor of Menden-Deuer & Lessard (2000).

For the size fractionation of chl *a*, 2 l of lake water was filtered in cascades over nylon (33 µm, 11 µm) and GF/F filters (Teubner & Dokulil 2002) and analysed according to APHA (1992) and Wright et al. (1991).

The persistence in the ciliate assemblage was measured as Bray-Curtis similarity between each pair of successive monthly samples. The similarity was based on continuous data, i.e. we considered species that represented >1% of the total abundance over seasons and depths (n = 192), respectively. Further, we calculated the net change (k_a) of ciliate abundance (a) with time (t) as:

$$k_a = \frac{\ln a_2 - \ln a_1}{t_2 - t_1}$$

Therefore, we used the same time intervals as for Bray-Curtis similarity. As most of the abundant ciliates (35 species) were not distributed normally (Kolmogorov-Smirnov), changes in the ciliate assemblage were analysed by non-metric multidimensional scaling (NMS, based on the standardised Bray-Curtis distance matrix), with 2 predefined dimensions to reduce the final stress of the ordination to 0.12 (Clarke 1993). Stress values explain the correspondence between the matrix and the final plot, i.e. '0' in the case of perfect concordance; <0.05 (5%), very good concordance; and between 0.05 and 0.30, reliable concordance. Changes were separated for time and depth along the first and the second NMS axis, respectively, and their relationship was analysed by Spearman rank correlations. Bray–Curtis similarity and NMS were carried out with the software packages PRIMER 5 and PCORD 4 for Windows, respectively.

Following the literature (Foissner et al. 1999), we catagorised the detected ciliates into 4 functional guilds, i.e. algivorous, bacterivorous, mixotrophic and omnivorous. The potential food resources were heterotrophic bacteria, 3 size fractions of chl *a* (<11 µm, 11–33 µm and >33 µm) and phagotrophic flagellates. The relationship between the protists and their potential food sources was analysed by stepwise multiple linear regressions (MLR) and Pearson's correlation after log-transformation (data transformed to normal distribution). The persistence of the ciliate assemblage over seasons and depths was analysed by notched box-whisker plots created in SYSTAT 8.0 for Windows. Boxes were notched at the median, with 95% confidence intervals. The length of each box corresponds to 50% of the values used for the analysis. Statistically significant differences between data sets are indicated by non-overlapping notches (equal to 95% confidence limit).

RESULTS AND DISCUSSION

Ciliates

The distribution patterns for ciliates over seasons and depths at the 3 sampling sites VI, EB and RB in Traunsee are shown in Figs. 1A & 2. Highest cell densities occurred in the top 30 m at all sites, including the euphotic zone, and in epi- and metalimnetic strata during summer stratification (Fig. 1A, Tables 1 & 2). Abundance and biovolume of ciliates were not significantly different between the 3 sampling sites from 0 to 40 m depths (Fig. 2E). We observed 3 peaks in abundance and biovolume, i.e. in March (with highest values at VI), in May at the time of the phytoplankton maximum and a major peak in autumn (Fig. 1A,

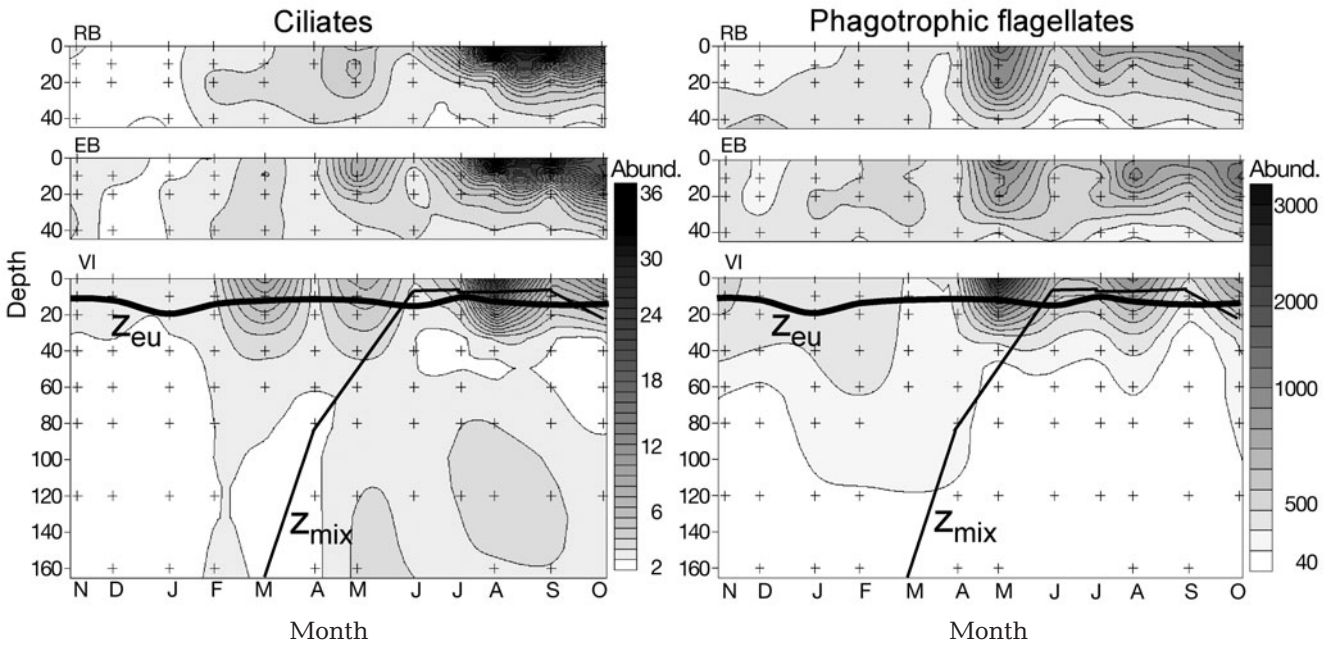

Fig. 1. Vertical and seasonal distribution of the abundance of ciliates (left panel, cells ml^{-1}) and phagotrophic flagellates (right panel, cells ml^{-1}) at the 3 sites (EB: Ebensee Bay; RB: Rindbach Bay; VI: Viechtau) in Traunsee from November 1997 through October 1998 (crosses: sampling dates and depths; lines: mixing depth [z_{mix}] and euphotic zone [z_{eu}])

Table 2, see also Sonntag et al. 2002). Long-term studies on mesotrophic lakes in temperate zones generally showed a bimodal seasonal pattern, with distinct peaks in spring (major peak) and in summer/autumn (Müller et al. 1991a,b, review of Laybourn-Parry 1994, Schweizer 1994, Pfister et al. 2002). Throughout the year, *Rimostrombidium brachykinetum*/*Rimostrombidium hyalinum* (not recorded separately), *Rimostrombidium humile* and *Balanion planctonicum* were the most abundant ciliate species in the surface waters, i.e. they accounted for 44% of the annual mean abundance and 15% of the annual mean biovolume, respectively (Table 2). Further quantitatively important ciliates were *Urotricha* spp. <50 µm, *Pelagohalteria cirrifera*/*Halteria bifurcata*, *Limnostrombidium pelagicum*, *Pelagostrombidium fallax*/*Pelagostrombidium mirabile* (not recorded separately due to taxonomical ambiguities—see discussion in Foissner et al. 1999), *Tintinnopsis cylindrata*, *Tintinnidium pusillum* and *Histiobalantium bodamicum*.

The relative contribution of the different ciliate groups to total ciliate abundance from November 1997 through October 1998 is shown in Fig. 3. The dominant group in the top 40 m was the Oligotrichida, followed by Prostomatida > Hymenostomata > Gymnostomatea/Peritrichia, while in the 60 to 160 m layers Hymenostomata dominated over Oligotrichida > Gymnostomatea > Prostomatida > Peritrichia. Predominance of oligotrichs ('Oligotrichetea' as defined by Foissner et al. 1991) has been reported earlier for oligotrophic lakes by Beaver & Crisman (1989) in Florida, James et al. (1995) for Lake Taupo in New Zealand and Félip et al. (1999) for the 70 m deep, alpine Lake Redó in the Pyrenees, Spain. As already shown elsewhere, the trophic status of a lake affects the composition of pelagic ciliate assemblages (e.g. Beaver & Crisman 1989, Pfister et al. 2002). In an investigation of several meso- to hypertrophic shallow lakes (<10 m depth) in northern Germany, Pfister et al. (2002) found that eutrophic lakes generally showed the most diverse ciliate assemblage in all seasons sampled, while mesotrophic lakes were dominated by oligotrichs and peritrichs in spring/summer and prostomatids and hymenostomatids in autumn. The dominant groups in hypertrophic lakes were oligotrichs, peritrichs, hypotrichs, heterotrichs and prostomatids. Under eutrophic and hypertrophic conditions high bacterial numbers support large assemblages of scuticociliates, mostly of the genera *Cyclidium* and *Uronema*, and oligotrichs, e.g. *Halteria* spp. (Nakano et al. 1998, Šimek et al. 2000).

In Traunsee, we identified around 60 pelagic ciliate species, and recent findings of Sonntag & Foissner (2004, unpubl. data) indicate the occurrence of several further still poorly or undescribed taxa. With depth, we followed the increase of various scuticociliates (1 probably undescribed species, *Histiobalantium bodamicum*, and others) and confirm the assumption of Müller et al. (1991b) that a decrease of ciliates with depth and low numbers of scuticociliates are characteristic for deep lakes with aerobic hypolimnia. In 2 neighbouring lakes,

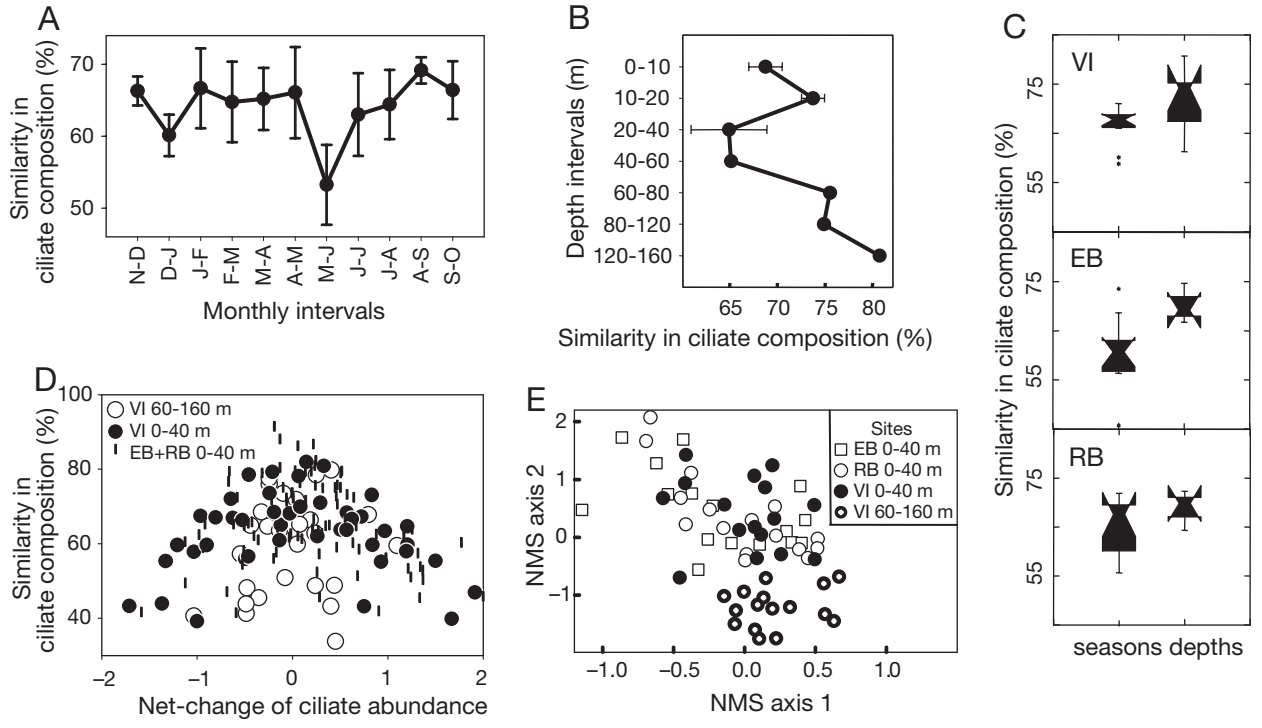

Fig. 2. Similarity in the ciliate assemblage between depths and seasons. (A) Similarity according to the Bray-Curtis index between successive monthly samples for the 3 sites (mean ± SD). (B) Same as Panel A for depths. (C) Similarity with seasons and along depths for each site as notched box-whisker plots. (D) Relationship between the monthly change in the ciliate species composition (Bray–Curtis similarity) and the monthly net change of the ciliate abundance for individual sites and depths. Sites EB and RB not labelled separately as their distributions are similar. (E) Non-metric multidimensional scaling analysis (NMS) of the 35 most abundant ciliate taxa. Each point represents the mean by season and depth for each sampling site: points were labelled by sites. The label overlay for seasons and depths for the first and the second axis, respectively, is shown in Fig. 4A,F. Stress: 0.12 (12%)

i.e. the oligotrophic Attersee and the oligo-mesotrophic Hallstättersee, we observed the same tendency (Sonntag et al. 2002). In Traunsee, the ciliate assemblage changed significantly with depth in relation to environmental parameters (Fig. 2B, Table 3). Furthermore, no anaerobic ciliates were found during the investigation period, which agrees with our observations that the whole water column was always oxygenated (Klammer et al. 2002). Occasionally, the main tributary, the Traun River, carried some single cyrtophorid ciliates into the lake. As the river water flows along the south-west coast of Traunsee, we detected such ciliates mainly at RB, but also at VI and EB.

Throughout the year, changes in algal and bacterial numbers led to specific ciliate successions (Figs. 4 & 5). Ciliate functional guilds were related to their potential food resources (Figs. 4 & 5, Tables 2 & 4). Throughout the year, total ciliate numbers were composed of around 90% algivores, 8% mixotrophs and 2% bacteri- and omnivores (Fig. 5C). The most predictive food sources for total, algivorous and bacterivorous ciliates were heterotrophic bacteria, on the one hand, and phytoplankton species in the size fraction of 11–33 µm, on the other hand (Table 4). This medium-sized phytoplankton fraction was composed of 36% diatoms, 26% cryptophytes, 22% chrysophytes, 9% chlorophytes and 7% dinoflagellates. Bacterivorous species occurred primarily in the deeper layers, from 60 to 160 m, where they accounted for around 50% of total abundance. A similar increase in abundance with depth was observed earlier by Taylor & Heynen (1987), Müller et al. (1991a) and Carrias et al. (1998a). The decrease in potential food sources, i.e. bacteria, phagotrophic flagellates and algae, from surface to deeper layers was significantly related to changes in the ciliate assemblage (Fig. 4F–J). Furthermore, the ciliate assemblage structure along depths was also significantly associated with depth gradients of temperature, conductivity and concentrations of dissolved organic carbon (DOC), total phosphorus, oxygen and chloride (NMS Axis 2; Table 3A). Food sources, however, were the primary factors driving the ciliate assemblage structure along the depth gradient (correlation coefficients > 0.81, $p < 0.01$; Fig. 4I–J) compared to environmental parameters (NMS Axis 2: correlation coefficients < 0.68, $p < 0.01$; Table 3A).

Table 2. Annual mean abundance of total and single ciliate species (cells l^{-1}), ciliate biovolume (mm^3 l^{-1}), free-living flagellates (10^6 cells l^{-1}), epiphytic flagellates (10^3 cells l^{-1}), heterotrophic bacteria (10^9 cells l^{-1}), picocyanobacteria (10^7 cells l^{-1}) and chl *a* (µg l^{-1}) in Traunsee from Nov 1997 through Oct 1998. Minimum and maximum values are given in brackets. Ciliates present below 0.2 cells ml^{-1} are recorded as 'single' findings. Black boxes indicate months with highest values (peaks), grey boxes the presence and white boxes the absence of the respective parameter. Values 0–40 m were depth and site integrated, values for 60–160 m integrated for VI only. Ciliates' preferred food or feeding mode are given as: a: algae, b: bacteria, m: mixotrophic, o: omnivorous, ?: food not known

	Depths (m)	Month N	D	J	F	M	A	M	J	J	A	S	O	Abundance or volume	Preferred food or feeding mode
Ciliate abundance (cells l^{-1})	0–40	■	■	■	■	■	■	■	■	■	■	■	■	4435 (235–36 574)	
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	1387 (320–2764)	
Ciliate biovolume (mm^3 l^{-1})	0–40	■	■	■	■	■	■	■	■	■	■	■	■	0.069 (0.015–0.973)	
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	0.009 (0.002–0.024)	
Free-living flagellate abundance (10^6 cells l^{-1})	0–40	■	■	■	■	■	■	■	■	■	■	■	■	0.6 (0.05–2.8)	
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	0.16 (0.04–0.34)	
Free-living flagellate biovolume (mm^3 l^{-1})	0–40	■	■	■	■	■	■	■	■	■	■	■	■	0.014 (0.005–0.085)	
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	0.006 (0.001–0.014)	
Sessile flagellate abundance (10^3 cells l^{-1})	0–40	□	□	□	□	□	□	■	■	■	■	■	■	5.8 (0–134.0)	
	60–160	□	□	□	□	□	□	□	■	■	■	□	■	2.0 (0–25.0)	
Bacterial abundance (10^9 cells l^{-1})[a]	0–40	■	■	■	■	■	■	■	■	■	■	■	■	1.4 (0.7–2.7)	
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	0.6 (0.4–1.1)	
Picocyanobacterial abundance (10^7 cells l^{-1})[a]	0–40	■	■	■	■	■	■	■	■	■	■	■	■	1.9 (0.2–9.9)	
Chl *a* (µg l^{-1})	0–20	■	■	■	■	■	■	■	■	■	■	■	■	1.1 (0.1–3.4)	
Oligotrichida (cells l^{-1})															
Codonella cratera (Leidy, 1877)	0–40	□	□	□	□	□	■	■	□	□	□	□	□	Single	a
Limnostrombidium pelagicum (Kahl, 1932)	0–40	■	■	□	□	□	□	■	■	■	■	■	■	121 (0–3833)	a
	60–160	□	□	□	□	□	□	□	□	□	■	■	□	Single	
Pelagohalteria cirrifera (Kahl, 1932)/ *Halteria bifurcata* (Tamar, 1968)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	367 (0–2223)	a
	60–160	□	■	■	■	■	■	■	■	■	■	■	■	142 (0–620)	
Pelagohalteria viridis (Fromentel, 1876)	0–40	■	■	□	□	■	□	□	■	■	■	■	■	151 (0–3153)	m
Pelagostrombidium fallax (Zacharias, 1895)/ *P. mirabile* (Penard, 1916)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	83 (0–3426)	m
	60–160	□	□	□	□	■	□	□	□	□	□	□	□	Single	
Rimostrombidium brachykinetum (Krainer, 1995)/*R. hyalinum* (Mirabdullaev, 1985)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	884 (47–10 548)	a
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	128 (16–360)	
Rimostrombidium humile (Penard, 1922)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	287 (0–4151)	a
	60–160	■	■	■	■	■	■	■	■	■	□	■	■	27 (0–161)	
Rimostrombidium lacustris (Foissner, Skogstad & Pratt, 1988)	0–40	■	□	□	■	■	□	■	□	□	■	□	■	30 (0–905)	a
	60–160	□	□	□	□	■	□	□	□	□	□	□	□	Single	
Tintinnopsis cylindrata Kofoid & Campbell, 1929	0–40	□	□	□	□	□	□	■	■	■	■	■	■	246 (0–12 153)	a
	60–160	□	□	□	□	□	□	■	■	■	□	■	■	4 (0–61)	
Tintinnidium pusillum Entz, 1909	0–40	□	■	□	■	■	■	■	■	□	■	■	■	88 (0–4248)	a
	60–160	□	□	□	■	■	■	■	■	■	■	□	■	8 (0–76)	
Prostomatida (cells l^{-1})															
Balanion planctonicum (Foissner, Oleksiv & Müller, 1990)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	611 (0–5446)	a
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	44 (0–158)	
Coleps spetai Foissner, 1984	0–40	□	□	■	□	■	■	□	□	■	■	■	■	42 (0–1318)	m
Urotricha spp. (at least 4 species)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	526 (0–6019)	a
	60–160	■	□	■	■	■	■	■	■	■	■	■	■	37 (0–185)	
Urotricha venatrix Kahl, 1935	0–40	□	□	□	□	□	□	□	□	□	■	■	■	9 (0–516)	o
Gymnostomatea (cells l^{-1})															
Askenasia chlorelligera Krainer & Foissner, 1990	0–40	■	■	□	□	■	■	□	■	■	■	■	■	72 (0–1813)	m
	60–160	□	■	□	□	□	□	□	□	□	□	□	□	Single	
Askenasia sp.[b]	0–40	□	□	□	□	□	□	□	□	■	□	■	■	114 (0–3888)	a
Askenasia volvox (Eichwald, 1852)/ *A. acrostomia* Krainer & Foissner, 1990	0–40	■	□	■	■	■	■	■	■	■	■	■	■	37 (0–344)	a
	60–160	□	□	■	□	■	■	□	■	□	□	■	□	3 (0–23)	

Table 2 (continued)

	Depths (m)	Month N	D	J	F	M	A	M	J	J	A	S	O	Abundance or volume	Preferred food or feeding mode
Monodinium chlorelligerum Krainer, 1995	0–40	□	□	□	□	□	□	□	□	■	■	■	■	41 (0–4217)	m
Mesodinium sp.	0–40	■	□	■	■	■	■	■	■	■	■	■	■	21 (0–172)	o
	60–160	■	□	■	■	■	■	□	■	■	■	■	■	41 (0–184)	
Paradileptus elephantinus (Svec, 1897)	0–40	□	□	□	□	□	□	□	□	□	□	■	■	2 (0–115)	o
Rhabdoaskenasia minima Krainer & Foissner, 1990	0–40	■	■	■	■	■	■	■	■	■	■	■	■	43 (0–351)	a
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	114 (0–344)	
Spathidium cf. *depressum* Kahl, 1930	0–40	□	□	□	□	□	□	□	□	□	□	□	■	Single	a
	60–160	□	□	□	□	□	□	□	□	□	□	□	■	Single	
Pleurostomatida (cells l^{-1})															
Enchelys gasterosteus Kahl, 1926	0–40	□	□	□	□	□	■	□	□	■	□	□	□	Single	o
Lacrymaria sp.[b]	0–40	□	□	□	□	□	□	■	■	□	□	□	□	Single	o
	60–160	□	□	□	□	□	■	■	□	□	□	□	□	Single	
Lagynophrya acuminata Kahl, 1935/ *Lagynophrya* sp.[b]	0–40	□	□	□	□	■	■	□	□	■	■	■	■	8 (0–213)	a
	60–160	□	□	□	□	■	□	□	□	□	■	□	□	Single	
Amphileptus piger (Vuxanovici, 1962)	0–40	□	□	□	□	□	□	■	■	■	■	■	□	Single	?
	60–160	□	□	□	□	□	□	□	□	□	■	□	□	Single	
Hypotrichia (cells l^{-1})															
Aspidisca cicada[c] (Mueller, 1786)	0–40	□	□	□	□	■	□	■	□	□	□	□	□	Single	a
Holosticha spp.[c] (at least 2 species)	0–40	□	□	□	□	■	□	□	□	□	□	□	□	Single	o
Uroleptus sp.[d]	0–40	□	□	□	□	■	□	□	■	□	□	■	□	Single	m
Hymenostomata (cells l^{-1})															
Cinetochilum margaritaceum (Ehrenberg, 1831)	0–40	□	□	■	■	□	□	■	□	□	□	□	□	Single	b
Histiobalantium bodamicum Krainer & Müller, 1995	0–40	■	■	■	■	■	■	■	■	■	■	■	■	268 (0–2035)	a
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	52 (0–246)	
Paramecium sp.	0–40	□	■	□	□	■	□	□	□	□	□	□	□	Single	b
Pleuronema sp.	0–40	□	□	□	□	■	□	□	□	□	□	□	□	Single	b
Scuticociliata[b]	0–40	■	□	■	■	■	■	■	■	■	■	■	■	21 (0–453)	b
	60–160	■	■	■	■	■	■	■	■	■	■	■	■	528 (64–1624)	
Scuticociliata (at least 4 species)	0–40	■	■	■	■	■	■	■	■	■	■	■	■	Single	b
	60–160	□	□	□	□	□	□	■	■	■	■	■	■	186 (0–988)	
Uronema nigricans (Mueller, 1786)	0–40	□	□	□	□	□	□	□	□	■	□	■	■	Single	b
Peritrichia (cells l^{-1})															
Epistylis sp.	0–40	■	□	□	□	□	□	□	□	□	■	□	□	12 (0–1299)	b
Pelagovorticella natans (Fauré-Fremiet, 1924)	0–40	■	■	■	■	■	■	■	■	■	■	□	■	18 (0–344)	b
	60–160	□	■	■	□	□	□	□	□	□	□	■	□	Single	
Pseudohaplocaulus infravacuolatus Foissner & Brozek, 1996 + *Vorticella chlorellata* Stiller, 1940	0–40	□	□	□	□	□	□	□	□	■	■	■	□	13 (0–1462)	a & m
Vaginicola ingenita (Mueller, 1786)	0–40	■	□	□	□	□	□	□	■	■	■	■	■	28 (0–344)	b
	60–160	□	□	□	□	□	□	□	■	■	□	□	□	5 (0–66)	
Vorticella vernalis Stokes, 1887	0–40	■	■	■	■	■	■	■	■	■	■	■	■	62 (0–602)	a
	60–160	□	■	□	□	□	□	□	■	□	□	□	□	Single	
Suctoria (cells l^{-1})															
Gajewskaiophrya melosirae (Gajewskaja, 1933)	0–40	■	□	□	□	■	□	■	■	■	■	■	■	8 (0–172)	o
	60–160	□	□	□	□	□	□	□	■	■	■	□	□	Single	
Cyrtophorida (cells l^{-1})															
Gastronauta membranaceus[c] Buetschli, 1889	0–40	□	□	□	□	□	□	□	□	■	□	□	□	Single	b
Pseudochilodonopsis fluviatilis[c] Foissner, 1988	0–40	□	□	□	□	■	□	□	□	□	□	□	□	Single	a
Cyrtophorida[c] (at least 2 species)	0–40	■	■	■	□	□	□	□	□	■	□	□	□	Single	a

[a] Data from Klammer et al. (2002)
[b] Probably new species
[c] Ciliates imported into Traunsee by the Traun River
[d] Description in prep.

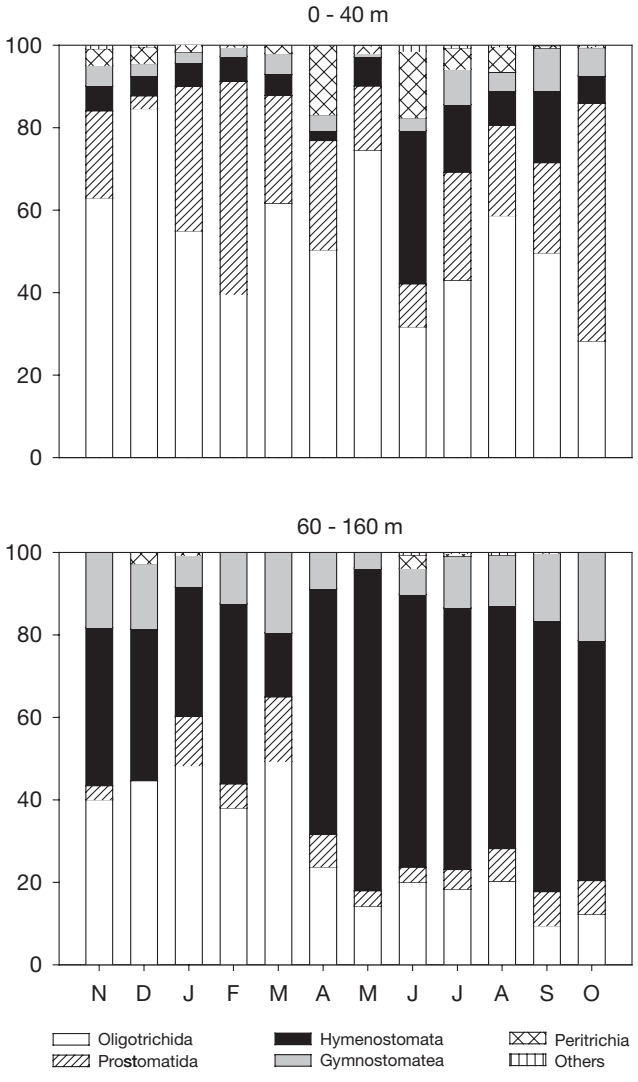

Fig. 3. Monthly percentages of the different ciliate groups at VI integrated over depths from 0 to 40 m (representative also for EB and RB) and from 60 to 160 m, respectively

The most remarkable change in the ciliate species composition along depths was found below both the euphotic and the mixing depth, i.e. between 20 and 40 m and 40 and 60 m, respectively (Figs. 1A & 2B). Changes in the ciliate assemblage structure were more persistent along depths than during seasons at all 3 sites (Fig. 2C). In particular, the persistence along depths was significantly higher at VI and EB than at RB. The more detailed similarity analysis by NMS showed that changes between months were less gradual, i.e. the relationship between the total abundance of ciliates and changes in the assemblage structure during seasons explained 66% of the similarity (Fig. 4A), along depths, 83% (Fig. 4F). The high percentage of algivorous ciliates explains the close

Table 3. Correspondence between environmental parameters and the ciliate assemblage structure changing with seasons and depths. The assemblage structure is summarised by scores of the first and second axis of NMS. The community structure along seasons and depths is summarised by scores of the first and second axis of NMS, respectively. (A) Relationship between environmental data and scores of the NMS axis (Pearson correlation coefficients) (DOC: dissolved organic carbon; TP: total phosphorus; O_2: oxygen). (B) Spearman rank order correlation between NMS axis and seasons, sites and depths (codes in brackets). Scores are displayed in Figs. 2E & 4A,F

	NMS Axis 1 (indicating mainly seasons)	NMS Axis 2 (indicating mainly depths)
(A)		
Temperature	−0.67**	0.68**
DOC	ns	0.55**
TP	ns	0.42**
O_2	ns	0.35**
Conductivity	ns	−0.56**
Chloride	0.33*	−0.66**
(B)		
Sites (coded as: 1 = VI, 2 = EB, 3 = RB)	ns	0.37**
Seasons (coded as: 1 = winter, 2 = spring, 3 = summer, 4 = autumn)	−0.67**	ns
Depths (coded with increasing numbers: 0, 10, 20, 40, 60, 80, 120, 160 m)	0.34**	−0.85**

relationship to changes in the total ciliate assemblage along depths (Fig. 4G). Mixotrophic ciliates followed the trend of algivorous ones (Fig. 4G). Both guilds were most numerous in the surface layers of Traunsee, decreasing with depth. Omnivorous ciliates followed the same vertical trend, however not significantly, and bacterivorous ciliates were inversely related to depth and increasingly important at deeper layers (Fig. 4H).

In the following, we describe in detail the pronounced changes of the ciliate assemblage structure during the 4 seasons in a functional model based on the mean annual abundance of the different nutritional modes in the top 20 m of the water column, as well as of the heterotrophic bacteria, phagotrophic flagellates and chl *a* (Fig. 5A,B). In addition, the relative contributions of the different nutritional modes are given according to season (Fig. 5C).

Seasonal succession of free-living ciliates and functional guilds

The seasonal succession of the whole ciliate assemblage was significantly related to the seasonal abun-

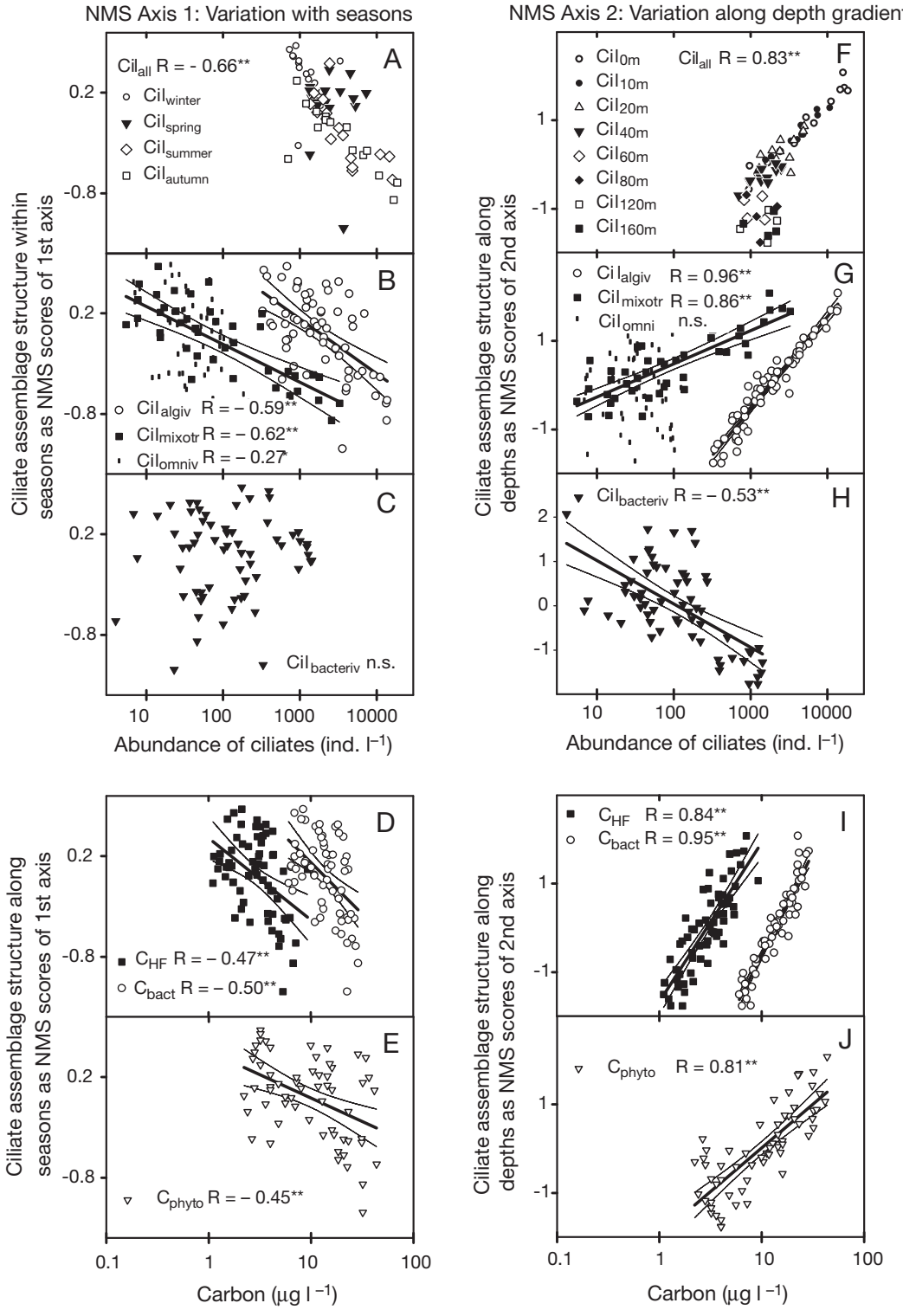

Fig. 4. Summary of the ciliate assemblage structure over seasons (scores of the first NMS axis, left panels) and depths (scores of the second NMS axis, right panels) related to the functional guilds of phagotrophic ciliates and flagellates, and bacteria and algae. Label overlay for seasons is shown in Panel A; for depths, in Panel F. Scores of the first NMS axis are most closely related to seasons; of the second axis, to depths (Spearman rank correlation coefficients and codes as in Table 3B). Relationship between NMS scores and abundance of total ciliates (A, F); algivorous, mixotrophic and omnivorous ciliates (B, G); bacterivorous ciliates (C, H); the carbon of phagotrophic flagellates and heterotrophic bacteria (D, I); and the carbon of phytoplankton (E, J) are shown. Pearson correlation coefficients are given (*$p < 0.05$; **$p < 0.01$; ns: not significant)

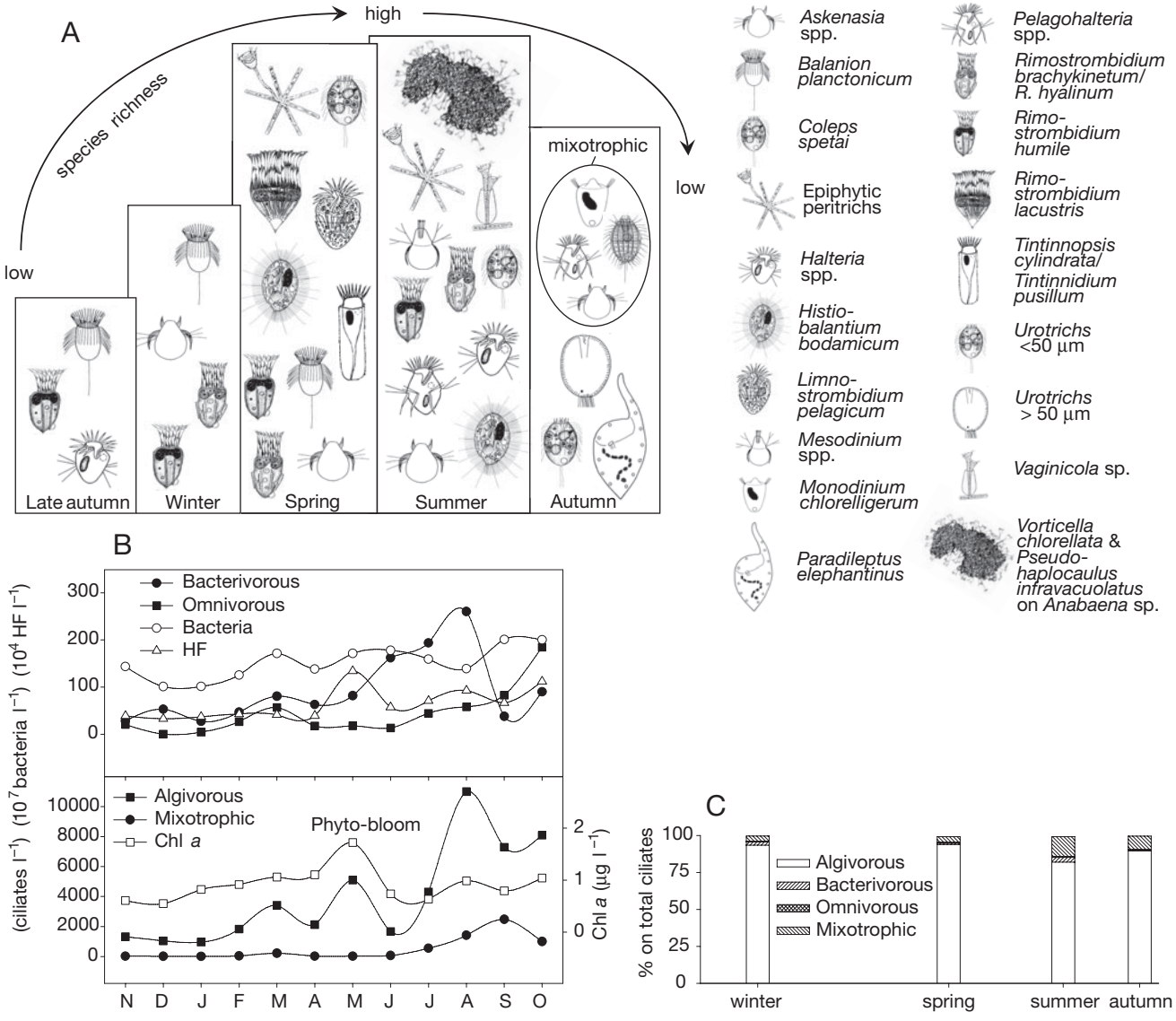

Fig. 5. Seasonal development of the ciliate assemblage for the upper 0 to 20 m. (A) Scheme of species richness with the dominant ciliate taxa shown (drawings from Foissner et al. 1999). (B) Mean monthly abundance of ciliates attributed to different nutritional modes (see 'Materials and methods'), phagotrophic flagellates (HF), heterotrophic bacteria and chlorophyll a (chl *a*). (C) Relative contribution of ciliate nutritional modes by season

dance of algivorous, mixotrophic and omnivorous ciliates and their potential food sources (Fig. 4B,D & E, respectively). Bacterivorous ciliates, however, were evenly distributed throughout seasons (Fig. 4C). Except for temperature and chloride concentration, the seasonal ciliate assemblage was not predictable by other environmental parameters (NMS Axis 1; Table 3A). The stability of the ciliate 'standing crop' was evaluated by the net change of the abundance (Fig. 2D). At all depth layers, >80% similarity in the ciliate assemblage between successive monthly samples was found associated with almost zero net changes of total biovolume. Conversely, a strong increase or decrease in the abundance of ciliates was associated with pronounced changes in the species composition (similarity < 50% between successive samples; Fig. 2A).

Winter

In winter (November to February) and during holomixis, only a few ciliate species were found, and these in low numbers, due to the poor nutrient situation and low temperatures (Figs. 1A & 5A,B, Table 2). In Decem-

Table 4. Stepwise multiple linear regression (MLR) for the control of multiple food resources (carbon) of ciliates and phagotrophic flagellates (R^2: squared correlation coefficient of MLP; coeff.: unstandardised coefficient [$^*p < 0.05$, $^{**}p < 0.01$, n.s: not significant]; SE: standard error of unstandardised coefficient; beta: standardised coefficient; algiv.: algivorous; bacteriv.: bacterivorous; mixotr.: mixotrophic; omniv.: omnivorous; HF: carbon of phagotrophic flagellates; na: not applicable)

Dependent variable		Independent variable entered										
		Carbon from heterotrophic bacteria		Carbon from phagotrophic flagellates		Carbon from chlorophyll (fraction <11 µm)		Carbon from chlorophyll (fraction 11–33 µm)		Carbon from chlorophyll (fraction >33 µm)		Constant
Group	R^2	Coeff. (SE)	Beta	Coeff. (SE)	Beta	Coeff. (SE)	Beta	Coeff. (SE)	Beta	Coeff. (SE)	Beta	Coeff. (SE)
Total ciliates	0.80	0.51* (0.22)	0.25	ns		ns		1.17** (0.19)	0.63	ns		5.62** (0.49)
Algiv. ciliates	0.97	1.59** (0.15)	0.65	ns		ns		0.76** (0.13)	0.36	ns		2.72** (0.34)
Bacteriv. ciliates	0.80	−3.49** (0.53)	−1.09	ns		−0.72** (0.27)	−0.39	2.65** (0.42)	0.96	ns		13.43** (1.09)
Mixotr. ciliates	0.83	ns		2.50* (1.12)	0.40	ns		2.12* (0.80)	0.47	ns		ns
Omniv. ciliates	0.26	ns		ns		ns		0.70* (0.35)	0.26	ns		2.82** (0.30)
HF	0.87	0.58** (0.09)	0.70	na	na	0.10* (0.05)	0.21	ns	ns	ns		−0.48* (0.18)

ber 1997, >80% of the ciliates were represented by oligotrichs, i.e. *Pelagohalteria cirrifera* and *Rimostrombidium* spp. (Figs. 3 & 5A,B). Towards the end of the winter, predominantly algivorous raptorial and filter feeders, such as *Balanion planctonicum*, *Rimostrombidium* spp., *P. cirrifera* and *Rhabdoaskenasia minima*, increased in abundance parallel to the development of chl *a*. In March, we noticed an exceptional peak of mainly algivorous and omnivorous ciliates at VI at depths of 10 to 20 m, presumably influenced by nutrient inputs by the Traun River during spring snowmelt. Correspondingly, peaks in picocyanobacteria, centric and pennate diatoms and cryptophytes were observed (data not shown). *B. planctonicum*, *Rimostrombidium* spp., *P. cirrifera*, *Mesodinium* sp. and *Urotricha* spp. responded numerically to this increase in food, even at temperatures of 4 to 5°C. The food vacuoles of, e.g., *Rimostrombidium lacustris* or *R. humile* were densely packed with centric diatoms. Müller and co-workers (Müller 1989, Müller et al. 1991b, Müller & Schlegel 1999) observed that the spring assemblage of Lake Constance (mainly *B. planctonicum* and *Rimostrombidium* spp.) was closely related to food resources and able to respond rapidly to a bloom of small phytoplankton.

Spring

In spring (March to May), algivorous and omnivorous ciliates, namely *Balanion planctonicum*, *Rimostrombidium* spp., *Urotricha* spp., *Tintinnopsis cylindrata*, *Pelagohalteria cirrifera*, *Limnostrombidium pelagicum* and *Histiobalantium bodamicum* appeared during the phytoplankton bloom and the chl *a* maximum (Fig. 5A,B, Table 2). At this time of the year, ciliates might be strong competitors with metazoan organisms, due to low generation times; hence, ciliates like *B. planctonicum* were assumed to be important contributors to the beginning of the so-called clear-water phase in meso- to eutrophic lakes, in addition to rotifers and other ciliates (Sommaruga & Psenner 1993, 1995). Generally, they were among the first grazers of algal biomass in spring, with a preference for cryptophytes (Carrick & Fahnenstiel 1989, Müller et al. 1991b, Müller & Schlegel 1999).

Summer

At the onset of the stratification from May to June (Fig. 1A), ciliate and phytoplankton abundance decreased (Fig. 5B). Concurrently, the ciliate species composition changed abruptly (low similarity of 55% for May to June; Figs. 2A & 5B). In June and July, prostomatids, hymenostomatids and peritrichs appeared in low numbers (Table 2). Predation by copepods and cladocerans might have been the reason for low total ciliate abundance during these months (Müller 1989, Stockner & Shortreed 1989, Carrick et al. 1991, Müller et al. 1991a, Laybourn-Parry & Rogerson 1993, Laybourn-Parry 1994, Jersabek pers. comm.). Free-living ciliates, namely *Pelagohalteria viridis*, halteriids and rimostrombidiids were most likely able to resist grazing pressure by their jumping behaviour (Gilbert 1994,

review of Jürgens 1994, Wickham 1995). In contrast, epiphytic ciliates gained an advantage by their attachment to diatom colonies, thus becoming inedible for zooplankton predators (Carrias et al. 1998b, detailed discussion see below). Most likely, *Balanion planctonicum* was outcompeted in June by other algivorous ciliates like *Histiobalantium bodamicum* and haptorids (*Askenasia volvox/Askenasia acrostomia*, *Rhabdoaskenasia minima*, *Mesodinium* spp.) or grazed, e.g., by rotifers like *Keratella cochlearis* (Weisse & Frahm 2002). Then again *B. planctonicum* increased in abundance in July and had its maximum in autumn. These findings are similar to those of Müller et al. (1991b) recorded from Lake Constance. Further, the scuticociliate *H. bodamicum* has been described as a 'superior competitor at relatively low algal food concentrations' (Müller & Weisse 1994), which might explain its constantly rising mean abundance from May through September.

Late summer and autumn

Throughout the summer months the number of coexisting species increased (Fig. 5A,B, Table 2). From August through October, a heterogeneous assemblage including many mixotrophic species appeared. The abundance of the zoochlorella-bearing species, e.g. *Pelagohalteria viridis*, *Monodinium chlorelligerum*, *Coleps spetai* and *Askenasia chlorelligera*, increased in surface layers of Traunsee to a maximum of about 9000 cells l^{-1} in September, which accounted for 25% of the total ciliate abundance at this time. Mixotrophic ciliates can reach up to 53% of the total abundance at certain times (Carrias et al. 1998a). Ciliates containing symbiotic algae are known to accumulate either in clear surface waters or near the oxycline (Hecky & Kling 1981, Pace 1982, Berninger et al. 1986, Müller et al. 1991a, Carrias et al. 1998a). Mixotrophy, in general, is often considered as an adaptation permitting exploitation of food-poor oligotrophic environments, but serves a variety of purposes (Dolan & Pérez 2000). The diversity of ciliates and food availability further increased in autumn, and the relationships between these 2 parameters became less clear, as various environmental factors might have enhanced the species diversity at this time (Müller et al. 1991a). At the end of the autumnal ciliate peak in Traunsee, large-sized predators, i.e. *Urotricha venatrix* and *Paradileptus elephantinus*, appeared and even had rotifers in their food vacuoles. However, bacterivorous and predatory ciliates seemed to be of minor importance when they occurred in summer and late autumn, as algivorous and omnivorous species were able to ingest around 43% of the primary production per year in Lake Constance (Müller et al. 1991a, Straile 1995).

Epiphytic ciliates

Sessile, bacterivorous peritrich ciliates appeared along with the occurrence of colonial algae, their preferred attachment sites, i.e. *Vorticella vernalis* on *Fragilaria crotonensis* and *V. ingenita* on *Tabellaria fenestrata* and *Dinobryon* spp. (Table 2). We observed their highest abundance in April, with *V. vernalis* as the dominant species. In autumn, this ciliate species appeared again together with *V. ingenita*. In August, the epiphytic *Pseudohaplocaulus infravacuolatus* was associated with *Vorticella chlorellata*, both attached to the chain-like colonies of the cyanobacterium *Anabaena lemmermannii*. Ruttner (1937) previously described the occurrence of 2 vorticellids on *Anabaena flos-aquae* in Traunsee, and Carrias et al. (1998a) found this association in Lake Pavin. However, in the meantime, *A. flos-aquae* has disappeared from Traunsee, indicating a shift to more oligotrophic conditions. Hunt & Chein (1983 and references therein) described vorticellids attached to *Anabaena* colonies as typical members of the ciliate assemblage of the Great Lakes. In general, epibiotic ciliates and flagellates have a major grazing impact on free bacteria, even when they are present in low numbers (Carrias et al. 1996). The attachment of peritrichs to colonial algae apparently provides a refuge from potential grazers, as they are too bulky to be ingested by zooplankton (Carrick et al. 1991, Carrias et al. 1996).

Seasonal succession of free-living phagotrophic flagellates

The seasonal dynamics of phagotrophic flagellates (HF) showed 2 peaks, one during the phytoplankton bloom in spring and another in autumn (Figs. 1B & 5B). Abundance and biovolume in the top 40 m were not significantly different between EB, RB and VI (Fig. 1B). Taxa <5 µm in length (fixed) accounted for 88 to 90% of total HF at depths from 0 to 40 m and 82% from 60 to 160 m (Sonntag et al. 2002). Those small HF were represented primarily by *Monas*-like cells and a so far unidentified HF with a decentral nucleus. An unidentified HF >5 µm with a kinetoplast increased numerically with depth. *Kathablepharis* sp., another HF >5 µm assumed to be mixotrophic, was also present at low densities. In total, numbers of HF in Traunsee were within the range reported for oligo- to mesotrophic water bodies (e.g. Carrias et al. 1998b, Sonntag et al. 2002), i.e. between 40 and 2900 ml^{-1} for the present study.

The flagellate assemblage structure changed during the transition from the phytoplankton spring bloom to summer, with small HF such as heterokonts and epiphytic flagellates becoming increasingly abundant, as

already reported for Lake Constance (Cleven & Weisse 2001). The time of the phytoplankton bloom was also the time of the highest bacterial numbers (Table 2). Free-living HF in Traunsee were significantly correlated to ciliates (Fig. 4D) and heterotrophic bacteria in the top 40 m (Table 4). Small heterotrophic flagellates, e.g. *Spumella* spp., are known to be efficient bacterivores, able to consume around half of the bacterial production (Weisse 1990, 1991, Simon et al. 1998). A coincidence of bacterial and HF densities and the predominance of HF <5 µm in length has already been reported for Lake Constance (Weisse 1990, 1991, Cleven & Weisse 2001), Mondsee (Salbrechter & Arndt 1994) and Lake Pavin (Carrias et al. 1998b). However, the relationship between HF and bacteria seems to be stronger in systems with low bacterial abundance compared to systems with higher bacterial numbers (Gasol & Vaqué 1993).

Epiphytic flagellates

Sessile flagellates were present in the whole water column (0 to 160 m depth) from May through October, appearing along with the occurrence of suitable attachment sites, i.e. colonial diatoms. They accounted for up to 7% of total HF in Traunsee and 9 to 11% in Lake Pavin (Carrias et al. 1996, 1998b). In Traunsee, epiphytic flagellates were mainly represented by a choanoflagellate of the genus *Salpingoeca* (for taxonomic considerations see Sonntag et al. 2000) attached to the diatom *Asterionella formosa*, and an unidentified epiphytic flagellate on the diatom *Fragilaria crotonensis*. In May, *Salpingoeca* sp. accounted for around 50% of total epiphytic flagellates; from June through August, an unidentified morphotype dominated the sessile assemblage. Then, *Salpingoeca* sp. again increased numerically in September and October, accounting for ~100% of epiphytic flagellate abundance. However, its occurrence was not restricted to late summer and autumn as previously described (Weisse et al. 1995, Cleven & Weisse 2001, Auer & Arndt 2002). Šimek et al. (2004) observed that at certain times *Salpingoeca* sp. is the most important bacterivorous species in the plankton.

CONCLUSIONS

Here, we showed that phagotrophic ciliates assigned to at least 4 different guilds and flagellates are important members of the planktonic food web in a cold, deep, oligotrophic lake, an environment previously thought to be unfit for many protists. Although present in low numbers, our data on the seasonal and vertical distribution demonstrate that the phagotrophic protists occupy a number of different ecological niches at various trophic levels (primary and secondary consumers). High taxonomic resolution was a prerequisite to interpret the findings precisely, and we would like to encourage researchers to use the high-standard methods available, especially for ciliate identification, e.g. QPS instead of Lugol's solution.

We assessed the strength of predictive relationships structuring the ciliate assemblage along the depth gradient by parameters of the bottom-up control cascade, i.e. by nutrients (phosphorus, DOC) and food sources from algae to bacteria and flagellates. Hence, the coherent correlation pattern of food and environmental variables predicting changes in the ciliate assemblage structure clearly mediated bottom-up cascades through the food web. The seasonal succession in the ciliate assemblage was also significant, but less predictable by food-source parameters in contrast to its depth distribution. Hence, the seasonal changes in the ciliate structure were not primarily mediated by bottom-up control. We assume that seasonal community structure in Traunsee was also driven by effects of the top-down control not studied.

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